Structure-Function Relationships in Dynamic Combinatorial Libraries

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Summary

In recent years, research on de-novo life and Darwinian evolution in the molecular systems has made important progress through the studies focusing on synthetic replicators. The work carried out in this thesis provides examples on how variations in building block design affect self-assembly and self-replication behavior in dynamic combinatorial libraries. We also report how different self-replicators affect the behavior of each other in multi-building-block systems.

Chapter 1 provides an introduction in which possible definitions of life are discussed, together with the main characteristics of living systems (like self-replication and metabolism) and an overview of recent developments on self-replicators based on peptides and nucleobases. Examples mainly include kinetically controlled self-replication and emergence of replicators under out-of-equilibrium conditions. A brief overview of systems chemistry and dynamic combinatorial chemistry is given, which are useful tools to study the emergence of complex molecules and to facilitate the understanding of key concepts to develop yet more complex systems. This includes recent examples developed by our group featuring a nucleation-elongation mechanism of self-replication in DCLs, exponential growth of peptide-based self-replicators and their diversification. Lastly, we discuss examples of supramolecular polymers following a seeded-growth mechanism which are similar to systems developed by our group.

In **Chapter 2** we report two peptide-based self-replicators that were utilized to synthesize supramolecular polymers with controllable size and composition. Our observations indicated that the nature of the polymers was strongly affected by the morphology of the sheared seeds. While triblock-fibers were formed from stacks of short seeds, diblock-fibers grew from single fiber seeds. In the last part of this chapter, we report our attempts to directly visualize the block fibers using electron microscopy by introducing halogens into building blocks.

Most of the building blocks that were developed in our group consist of an aromatic core with two thiol units, a beta-sheet forming short peptide of which the first amino acid acts a spacer. In **Chapter 3** we show how self-replication can be tuned by the length of the spacer in DCLs made from two structurally different building blocks in which β-alanine and γ-aminobutyric acid were incorporated as spacer. After modifying the building blocks with these amino acids which contain additional methylene units compared to the original glycine spacer, we investigated the self-replication behavior of mutants in different conditions. We showed the emergence of differently sized self-replicators from the mutants of the first building block and inhibition of the self-replication in DCLs made from the mutants of the second building block.

Using a similar approach, in **Chapter 4** we discovered parasitic behavior between self-replicators formed from building blocks that differ by a single methylene unit. We observed that a 6-ring replicator can only emerge when assisted by a pre-existing 8-ring self-replicator. While the 8-ring templated the formation of hexameric species from one end of the fiber, it was degraded from the other end to the point that it was no longer detectable by the end of the experiment. We also provided experimental proof for the uni-directional cross-catalysis between the self-replicators: only 8-ring can cross-catalyze the formation of 6-ring species but not vice-versa.

Aiming for diversification of self-replicators through a cross-catalytic pathway under out-from-equilibrium conditions, we used continuous flow-set-ups in **Chapter 5**. By employing the first set of building blocks that were studied in Chapter 3, we infused mixtures of monomer, trimers and tetramers with varying building block compositions into a solution containing self-replicators and monitored their adaptation in time. First, we set up a system with two building blocks and optimized the experimental conditions in terms of flow rate, time and starting replicator composition. We also report attempts to create a cross-catalytic cycle by introducing a third building block. We observed a switch in the size of replicators over time. However, for a more detailed kinetic analysis, further optimization of analytical methods is required.

Finally, in **Chapter 6**, our studies are placed in a broader perspective and future prospects are discussed.